

GEOPHYSICAL VARIABLES AND BEHAVIOR: XCI. AMBULATORY BEHAVIOR IN RATS FOLLOWING PRENATAL EXPOSURES TO COMPLEX MAGNETIC FIELDS DESIGNED TO INTERACT WITH GENETIC EXPRESSION¹

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Summary.—A total of 45 litters were exposed during their entire prenatal development to one of two complex patterns of magnetic fields whose strengths varied within one of four intensity ranges between 10 nT and 1000 nT or to sham-field conditions. The litters exposed to the most complex pattern, composed of 50 200-msec. presentations of different pulses for 10 sec. every 50 sec., displayed more ambulation in an open field at 21 days of age than the litters that had been exposed continuously to a repetitive frequency-modulated field or to sham-field conditions. This treatment explained 25% of the variance in the numbers of squares traversed. The results suggest that complexity of the applied magnetic field during prenatal development may be more important than intensity for permanently affecting neuronal organization and behavior.

Alterations within the cytoplasm and nucleus of cells due to changes in the extracellular environment are assumed to be mediated by the interactions between sequestered ligands and their receptors within the cell membrane. However, the consequences of these interactions may be primarily mediated by more fundamental electromagnetic and ionic phenomena, such as calcium waves (Berridge, 1997) that contain the information to which the nucleus of the cell responds. The information mediated by these waves is related to their frequency, phase modulation, or pattern complexity rather than to their intensity (Hajjar & Bonventre, 1991; Tang & Othmer, 1995). Gene expression due to changes in the activity and the interactions between DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), manifested as transcription and the temporal arrangement of amino acid sequences (translation), can be dependent upon these modulations and complexities (Dolmetsch, Xu, & Lewis, 1998; Li, Llopis, Whitney, Zlokarnik, & Tsien, 1998).

Both theoretical and empirical research suggest that complex, weak (less than 1 microTesla) magnetic fields could mediate their effects by directly modifying the periodicity of these intrinsic cytoplasmic processes (Lednev, 1991; Liburdy, Callahan, Harland, Dunhan, Sloma, & Yaswen, 1993). This possibility indicates that the appropriate complex pattern of magnetic fields,

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one of the few natural stimuli penetrating living systems, could imitate the effects normally evoked by the interactions between natural ligands or pharmacological agents and their receptors. If the information (analogous to the genetic code) within these complex fields was the critical requirement for an effect, then the argument that strong homogeneous intensities of the applied fields are required to compensate for thermal agitation would be less applicable.

A number of patterns of magnetic fields with different temporal configurations has been shown to evoke mild to moderate shifts in pain thresholds (Fleming, Persinger, & Koren, 1994) and to affect learning (Richards, Persinger, & Koren, 1993, 1996; Stewart & Persinger, 2000). However, we reasoned that these different pulses would be analogous to the different letters of a bioelectromagnetic alphabet. To produce more cellularly meaningful effects within the cell, combinations of these pulses to form the electromagnetic words or combinations of these words to produce electromagnetic sentences would be required.

We also assumed that meaningful aggregates of electromagnetic patterns would require less energy or intensity to affect complex processes within the cell. As hypothesized by O'Connor and Persinger (1999) and shown by Sandyk (1993, 1995) even picoTesla and nanoTesla field strengths could be effective in the brain and body. In an analogous manner, a sentence can be whispered at 30 dB or screamed at 80 dB; however, the information contained within its syntax and semantics is not substantially altered. Comparable complexity in the temporal pattern of electromagnetic fields should be required to influence processes associated with gene expression.

Although the equivalent of the Rosetta stone (that allowed the deciphering of the Egyptian hieroglyphics) for the translation of electromagnetic patterns into their chemical equivalents has not been isolated, the present experiment was designed to test the feasibility of pursuing this approach. We decided to model the conceptual organization of the digital (base pair) system by which DNA and RNA determine the sequence of the chains of amino acids composing the proteins for the structure and function of the cell and organ. Consequently we set the pixel duration of each point that composed the computer-generated shapes to be 1 msec., the duration of each complex pattern to be 200 msec., and the total time for each package of pairs of complex patterns to be 3.2 sec. For comparison, the time required for the stacking of a base pair in a RNA sequence is about 1 msec., while the time required to incorporate an amino acid during protein synthesis is in the order of 10 msec. The time associated with the assembly of an entire protein is about 15 sec. (Maaloe, Maaloe, & Kjeldgaard, 1966).

As shown in Table 1, we designed an electromagnetic sequence composed of three different clusters of pairs of single complex patterns. The

pairs of patterns were called burst-firing and frequency-modulated or Thomas Pulse (Thomas, Kavaliers, Prato, & Ossenkopp, 1997), positive ringing and negative ringing, and fibonacci and sinusoid 5 Hz. The shapes of the first four patterns have been published elsewhere (Richards, *et al.*, 1993; Persinger, Tiller, & Koren, 2000) whereas the sinusoidal 5-Hz wave was generated from a sine wave function. The fibonacci sequence was composed of a pattern whose interpeak intervals increased as a function of the fibonacci series, e.g., 1 msec., 2 msec., 3 msec., 5 msec., 8 msec., 13 msec., until the sum of the intervals and pixel duration equalled 250 msec.

TABLE 1
CONTINUOUS TEMPORAL SEQUENCE OF DESIGN OF COMPLEX PATTERNS*

1.	Burst	LTP	Thomas	LTP	Burst	LTP	Thomas	LTP
2.	Burst	LTP	Thomas	LTP	Burst	LTP	Thomas	LTP
3.	Neutral							
4.	P-ring	LTP	N-ring	LTP	P-ring	LTP	N-ring	LTP
5.	P-ring	LTP	N-ring	LTP	P-ring	LTP	N-ring	LTP
6.	Neutral							
7.	Fibonacci	LTP	5 Hz	LTP	Fibonacci	LTP	5 Hz	LTP
8.	Fibonacci	LTP	5 Hz	LTP	Fibonacci	LTP	5 Hz	LTP

*The duration of each of the 50 presentations was 200 msec.

The simple LTP (long-term potentiation) pattern was employed as the analogue of the physical space between successive base pairs or, employing a linguistic metaphor, the space between letters. Because the LTP pulse appears to affect the representational traces associated with memory when applied as a magnetic field and amplify long-term potentiation within slices of hippocampal tissue when induced as electrical currents (Stewart & Persinger, 2000), it was employed to produce contrast enhancement of the effects of the previous pattern. Each of the three clusters of different pairs of complex patterns was designed to be presented four times followed by a neutral field. The total duration of the electromagnetic sentence or "sequence" was 10 sec. We also appreciated the nature of nuclear transcription whereby groups of three spatial patterns (nucleotide) code for a specific amino acid. In the present design the triad of patterns, e.g., burst-LTP-Thomas, etc. has also a potential configuration.

In the tradition of teratology, we selected the developing fetus as the system to be affected. We assumed that these biochemical processes within which there are strong spatial and temporal gradients, such as migrating waves of neuronal fields, would be most likely affected by the information within the applied magnetic fields. The quantitative amount of ambulation within the open field was selected as the primary measure because it has been employed as an inference of exploration in animals (Ossenkopp & Maz-

manian, 1985), one of the most important qualities for adaptation and survival.

METHOD

Subjects

A total of 45 litters of 21- to 22-day-old Wistar albino rats served as subjects. Their parents had been obtained from Charles River Breeders. Litters were employed as subjects rather than individuals because we assumed that each litter was functionally and statistically $n = 1$ rather than $n = 8$.

Procedure

In six separate blocks of experiments 48 female, 90- to 100-day-old Wistar albino rats and 24 male, 90- to 120-day-old male Wistar rats were housed in one of four equally sized compartments within the same plastic cage whose dimensions were 140 cm by 33 cm by 33 cm. There were two females and one male per compartment. At each end of the cage was a magnetic coil. The two coils were identical and were created by wrapping plastic cartons (milk crates) with 305 m of 30 AWG (Belden No. 9978) wire in a single layer (18 cm wide). The crates had been obtained from FREM Corporation (No. 701-4) and were 38 cm $\times$ 33 cm $\times$ 27 cm. The circuitry was designed so that only one coil would be activated for any given block of experiments.

Three of the blocks involved the employment of the complex pulse presented in Table 1. This sequence was presented for 10 sec. every 50 sec., i.e., 10 sec. on, 40 sec. off. This "mark-space ratio" for the presentation of the "electromagnetic sentence" was selected on the basis of our theoretical work that suggests that the ratios of relationships and the forms of temporal patterns are similar across levels of physical discourse although their absolute durations differ, i.e., temporal and spatial invariance (Persinger, 1999). Persinger, Cook, and Koren (2000) have employed this concept to develop potential treatments for demyelinating diseases, such as experimental allergic encephalomyelitis. In this latter instance, a complex, amplitude-modulated pattern of a 7-Hz square wave magnetic field between 10 and 50 nT was presented for 6 min. once per hour only during the scotophase.

The other three blocks employed the continuous presentation of the Thomas (frequency-modulated) pulse where the pulse durations were 3 msec. For two of the blocks for each pattern the activated coil was at one end of the cage while for the other block the other coil was activated. This allowed control for possible unknown artifacts associated with any of the four compartments. Because there was no statistically significant difference in the compartmental effects as a function of distance from either of the activated coils, the data were analyzed as a function of the distance from the activated coil.

The specific patterns of the magnetic field were generated by converting lines of numbers, each containing a number between 0 and 255 into voltages using an XT personal computer. There were 9,999 lines for the gene pulse and 849 lines for the Thomas pulse. These numbers were then converted by a custom-constructed device into voltages that were directed towards one of the two coils. Other details have been published previously (St-Pierre, Persinger, & Koren, 1998).

The two patterns, Thomas pulse and gene pattern, were initiated upon introduction of the triad of rats into the compartments and continued until the pups were delivered. To avoid competition between the two mothers, the second female was added to the compartment about five days after the first male and female were introduced. The males were removed 10 days after the beginning of the experiment (five days after the introduction of the second female). We assumed five days were sufficient for the occurrence of one oestrus required for impregnation. During the experiment Purina rat chow and water were available *ad libitum*. The light-dark cycle was 12:12 with the scotophase beginning at 2000 hr. Corncob bedding was removed and replaced once every two to three days during which time the rats were outside of the field for about 4 min.

According to measurements by instrumentation reported previously (St-Pierre, *et al.*, 1998) the ranges of intensities of the complex magnetic fields within the four chambers as a function of distance from the activated coil decreased exponentially. The four successive ranges, from either activated coil, were 590 nT to 1150 nT (11.5 mG), 90 nT to 180 nT, 30 to 50 nT, and 5 nT to 20 nT. The means and standard deviations for the intensities over any given 100-sec. interval (with the field present) for the four successive areas were 510 and 170 nT, 93 and 25 nT, 32 and 6.5 nT, and 21 and 5 nT, respectively. The light intensity from overhead fluorescent lamps was approximately 500 lux at the floor of the exposure cages. Care was taken to ensure that extraneous sources of power-frequency magnetic fields, such as those from clocks and computers, were distant from the cages, or that stray magnetic fields through the walls from other experimenters within the colony were not influential.

When the litters were born, they were removed within 24 hr. and culled to four male and four female pups. The litters and their mothers were placed in separate cages in another room. A total of 15 (of 24) were delivered during the three blocks of exposure to the frequency-modulated field and a total of 18 litters (of 24) were delivered during the exposure to the gene-sequence magnetic field. With the exception of the highest intensity groups exposed to the frequency-modulated pattern ($n=3$ litters), there were four to five litters per intensity condition. For comparisons, six litters exposed to sham conditions (same cage conditions but no field) and six litters

employed as controls (delivered in another room for a different experiment) were included in this study. Because there was no statistically significant difference in ambulatory behavior between these two groups, these 12 litters were combined into the sham-reference group.

On the day the litters were weaned, each pup was removed from its mother, carried in a cage to the test room (about 30 sec.) and observed within a closed, white, open field that was marked with 16 squares (Persinger, 1969). The luminosity at the floor of the field was between 1200 and 1300 lux, supplied by overhead fluorescent lamps. The numbers of squares traversed within 2 min. was measured for each rat. The experimenter who tested the rats had assumed that the effect from the pattern depicted in Table 1 would be intensity-dependent and was blind to the hypothesis that transfer of information would depend upon complexity and less on intensity.

The means for the numbers of squares traversed for the males and the females (separately) for each litter as well as the coefficients of variation (standard deviation divided by the mean) for the males and females from each litter were calculated. The latter measure was employed to discern the possible differential response of individual rats within a litter to the treatments. Three-way analyses of variance of the ambulatory score and the coefficient of variation as a function of pattern (Thomas pulse vs gene pulse), intensity (four levels), and sex were completed. Two-way analyses of variance with one within-subject measure (sex, i.e., males and females of the same litter) and treatment (Thomas pulse, gene pulse, and sham/reference group) were also completed. All analyses employed SPSS software.

RESULTS

The three-way analysis of variance as a function of sex (within-subject measure), pattern, and intensity demonstrated a statistically significant difference in ambulatory activity as a function of the pattern (Thomas vs gene) of the magnetic field to which the rats had been exposed during prenatal development ($F_{1,25} = 12.54$, $p < .001$; $\eta^2 = .33$). There were no statistically significant differences with respect to the intensity of the exposure ($F_{3,25} = .75$, $p > .05$), sex ($F_{1,25} = .41$, $p > .05$), or any of the interactions (all $F_s > 1.00$).

The means and standard deviations for the average (mean) numbers of squares traversed in the open field for 2 min. for the males and the females as well as the coefficients of variation for these measures are shown in Table 2. Two-way analyses of variance indicated a statistically significant main effect for ambulation ($F_{2,42} = 7.15$, $p < .01$; $\eta^2 = .25$). There was no statistically significant sex difference or interaction between sex and treatment. *Post hoc* analysis indicated that the major source of the main effect was the greater ambulation of the litters that had been exposed during prenatal development to the gene-pulse pattern compared to those exposed to the frequency-

TABLE 2
MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF SQUARES TRAVERSED IN OPEN
FIELD AND COEFFICIENTS OF VARIATION FOR VARIOUS TREATMENTS

Measure	Sham/ Reference		Frequency Modulation		Gene Sequence		η^2
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	
Ambulation							
Males	25.2	9.9	22.2	11.3	36.7	12.5	25
Females	26.7	13.6	23.5	10.0	37.4	15.0	21
Coefficient of Variation							
Males	68.4	21.4	91.1	26.7	50.0	27.2	34
Females	74.7	52.3	73.3	25.3	53.7	27.3	

modulated field or to the sham/reference conditions. The latter two groups did not differ significantly from each other.

The means and standard deviations for the coefficients of variation for the males and females within the litters exposed to either the frequency-modulated or gene pattern and to each of the four intensities are shown in Table 3. The results of the three-way analysis of variance of the coefficients of variation demonstrated a statistically significant difference between the patterns ($F_{1,25}=21.53$, $p<.001$; $\eta^2=.46$) that was due to the greater variation within the litters exposed prenatally to the Thomas pulse. All other main effects and interactions were not statistically significant except for the interactions between pattern and sex ($F_{1,25}=6.83$, $p<.01$; partial $\eta^2=.22$) and intensity (regardless of pattern) and sex ($F_{3,25}=3.34$, $p<.05$; partial $\eta^2=.29$).

TABLE 3
MEANS AND STANDARD DEVIATIONS FOR WITHIN-LITTER VARIABILITY IN OPEN-FIELD BEHAVIOR
FOR MALE AND FEMALE RATS AS A FUNCTION OF INTENSITY OF THE PATTERNS

Measure	Low-Low		Low		Medium		High	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Males								
Thomas Pulse	73 ^a	19	83 ¹	16	131 ^b	27	86 ^a	10
Gene Pulse	40	14	39	37	68 ^b	23	34 ^a	14
Females								
Thomas Pulse	86	36	65	28	79	14	65	16
Gene Pulse	58	12	52	36	57	36	45	9

Post hoc t tests indicated that the major source of the interaction between pattern and sex was due to the greater within-litter variation of the males exposed prenatally to the Thomas pulse compared to the within-litter variation of the males exposed to the gene pulse. *Post hoc* comparisons showed that the major source of the interaction between sex and intensity, regardless of pattern, was due to the greater within-litter variability for the

males that had been exposed to the 90 to 180 nT (0.9 mG to 1.8 mG) intensities compared to the females who did not display this particularly non-linear intensity effect.

DISCUSSION

The results of this study indicated rats exposed during their entire prenatal development for 10 sec. to a very complex magnetic field and then to no experimental field for 40 sec. displayed more ambulatory activity than did rats exposed to either a frequency-modulated field with comparable intensities or to sham fields. The effect was similar for both male and female rats and was sufficient to explain about one-quarter of the variance in ambulation within an open field.

The comparable magnitude of this increased ambulatory effect for litters exposed to the very weakest intensity (10 to 20 nT) to the strongest intensity (about 1 microT) suggests that information within the field or the temporal structure of the applied magnetic field may be more important than intensity at least within this range. Although intuitively the intensities of only 5 to 20 nT may appear as too weak to be bioeffective, such variations in geomagnetic activity for only several hours (compared to the entire gestation of 21 days) have been associated with enhanced seizure activity in rodents (Michon & Persinger, 1997), altered homing behavior in birds (Southern, 1971), and modifications of general ambulatory behavior in many species (Persinger & Lafreniere, 1977).

That the effect was an artifact of experimenter bias is unlikely because the experiment was conducted under blind conditions. Because the scores for the rats exposed to the sham field, routine colony room environment, or the frequency-modulated field did not differ from each other but all differed significantly from the scores for the rats that had been exposed to the gene-sequence field, the probability is greater that the observed experimental effect upon ambulation was from the exposure to the gene pulse.

When the developing fetus is exposed to complex magnetic fields some effects would be expected that suggest the operation of traditional physical models such as cubic ("window") or linear ("dose-dependent") relationships. In fact, the coefficient of variation did reflect a "window" of sensitivity for the male rats for both field patterns, particularly the Thomas pulse. The *post hoc* analysis indicated that the rats exposed to intensities between 0.9 mG and 1.8 mG, which would be similar to those generated most frequently by power frequencies within the bulk of living space occupied by human beings, was associated with a specific effect upon males relative to females. The increased coefficient of variation indicated that some males were more affected than others within the same litter and the same exposure intensity. The possible source for this anisotropy is not clear at present but reiterates the

importance of considering individual sensitivity of the developing organism to the effects of complex magnetic fields whose intensities have been considered nonconsequential according to traditional mechanisms.

This experiment, to our knowledge, is the first study to explore a new paradigm by which the interactions between weak complex electromagnetic fields and living systems might be understood. It involves the sequencing or chaining of complex magnetic fields into temporal patterns and periods that could be analogous to the coding systems and the timing associated with transcription and translation within the developing organism. To what extent the *optimal* combination of these sequences can represent organismic information that is similar in potency and contained within the sequences of DNA and RNA that determine gene expression as inferred by amino acid sequences (proteins) must still be explored.

The pursuit of these interactions should be considered urgent from an epidemiological perspective. Complexities, comparable to those produced in this study, are becoming routine components of the environmental electromagnetic matrix within a society of growing dependence upon wireless communication technology and spatial proximity to electromagnetic sources generated by computer systems. Ultra-wideband electromagnetic pulses between the Hz to GHz range, even with durations of a few nanoseconds, can deliver effective (temporally averaged) field strengths comparable to those employed in this study. Exposures of a few tens of minutes to these pulses can influence nitric oxide synthase and other reactions fundamental to the function of cells (Seaman, Belt, Doyle, & Mathur, 1999).

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